Leading article

Molecular biology of neurological diseases

W.J.K. Cumming

Withington Hospital, West Didsbury, Manchester M20 8LR, UK

Picture the scene. Neurologist on the telephone to a Departmental Librarian on a Friday morning. Neurologist: 'Has this week's *Nature* arrived?' Librarian: '*Nature*. I thought you said it was the Department of Neurology!'

The last 5 years have seen a major transformation in neurology, particularly in the understanding of those disorders where inheritance is either autosomal dominant, recessive or X-linked. Collaboration between Departments of Molecular Genetics and Neurology has developed to a point where gene markers for many of these disorders have now been identified.

Although inherited neurological diseases are by no means the commonest conditions seen in the average neurology clinic, the unravelling of the aetio-pathogenesis using the techniques of molecular biology^{1,2} has been scientifically intriguing and exciting in its own right but also is offering clues to the basic mechanisms underlying neurological³ and in particular neuromuscular disorders.⁴

As is usually the case, any sort of information explosion spawns its own literature and terminology which is often sufficient to frighten off even the most dedicated academic neurologist. Because the work is carried out in the field of basic science, information frequently appears as letters to *Nature* or *Science* and therefore to have any hope of keeping up to date, neurologists now have to have access to journals which they probably thought they would last consult when they were working for their M.D.!

The steps involved are however quite simple, and are: (a) chromosome localization and linkage; (b) fine mapping; (c) identification of gene mutation(s); (d) function of the gene and (e) rational therapy. For the majority of neurological disorders, studies are currently at phases (a) or (b); a few are getting towards (c); one is at (d) and potentially one is

getting towards (e). Clearly localization of the abnormal chromosome is easy in the X-linked disorders (e.g. Duchenne/Becker disease); however, where does one start to look for the chromosome involved in, for example, myotonic dystrophy?

Throughout the genome, there are well recognized fragments of DNA whose chromosome localization is precisely known (e.g. Duffy blood group, chromosome 1 and MHC on chromosome 6). Various other markers have been identified in the autosomes (about 700 to date) and the first step in analysis is to look for linkage between the known chromosome markers and the family in whom the disease has been inherited.⁵ Such linkage is said to be found when the LOD score (LOD being the logarithm of the probability ratio of the known marker co-segregating with the putative disease marker) is greater than 3 (or odds of 1000:1).

Once the chromosome has been localized either to its short (p) or long (q) arm then fine analysis of the DNA in these segments can be undertaken. Restriction fragment length polymorphisms (RFLPs) are short segments in DNA which can be produced by cleaving DNA at known sites (by restriction endonuclease). These fragments of DNA move in the same relation to each other in chromosome exchange and therefore linkage again is carried out at a single chromosome level and the chromosome is therefore 'walked' between known marker points in the hope of finding a marker which is tightly linked to the inheritance within the family (cf. the linkage of myotonic dystrophy on chromosome 19 to the site p13–15, that is in relation to the gene for creatine kinase MM).^{6,7}

This technique of linkage analysis with RFLPs leads to the development of flanked markers, that is, identified markers on either side of the abnormal gene, further narrowing the area which has to be screened to identify the actual abnormality of the gene itself. It is to this level that the majority of neurological disorders has now progressed. For example Charcot Marie Tooth disease (hereditary motor sensory neuropathy), is identified with markers on two chromosomes, 1 (HMSN 1b) and 17 (HMSN 1a).^{8,9} Neurofibromatosis (type 1;

Correspondence: W.J.K. Cumming, B.Sc., M.D., F.R.C.P.I., F.R.C.P.

Received: 31 October 1991

generalized) is linked to chromosome 17.¹⁰ The location of type 2 (acoustic neuroma and meningioma) is not yet known.¹¹ At present there is a great research push to identify the gene products in these disorders, since in particular in von Recklinghausen's disease, the disorder is known to be somewhere close to the gene related in nerve growth factor (NGF) which obviously has major implications well outside the restricted field of von Recklinghausen's disease itself.^{12,13}

Although these disorders are all at the rarer end of the spectrum of neurological conditions, in the fields of dementia and neuromuscular diseases, the developments in molecular biology and genetics are throwing up potentially fundamental changes in our understanding of the disorders. Huntington's disease was the first inherited neurological disorder to be located to a chromosome (4p),¹⁴ but despite something like 8 years of intense research, it has been impossible to identify a flanking marker and attempts at identifying the gene product have to date been unfruitful.¹⁵

However, in the field of Alzheimer's disease,¹⁶⁻²¹ the fact of inheritance has been known for many years and in very elegantly designed collaborative studies between departments of neurology and genetics, large pedigrees with inherited Alzheimer's disease have been identified. The chromosome localization is on 21 and fine analysis shows that the gene in these families is closely allied to the chromosome site for amyloid precursor protein,²² which has been considered, from the view point of neuropathology, to be one of the pivotal factors in the development of the classical pathological changes in Alzheimer's disease.

Motor neurone disease (amyotrophic lateral sclerosis, ALS) exists in a familial form and by using the techniques as described above, the chromosome location for familial MND has been identified (21)²³ and this raises the very exciting possibility of eventually being able to understand the fundamental pathogenesis of the condition even in the common situation of the sporadic case.

In the field of neuromuscular disease, Duchenne and Becker muscular dystrophy, considered collectively, are the commonest childhood neuromuscular disorders and here the major impact of molecular biology is best seen.^{24,25} Clearly researchers were at an advantage knowing that the disease had to be on the X-chromosome and through the mid-80s various flanking markers were identified which greatly enhanced our ability to identify carriers of the condition. In 1987 Kuncle and his colleagues²⁶ identified what they considered to be a candidate gene using the technique of chromosome walking with multiple RFLPs. The area of the gene they identified was considerable, containing about 2 million base pairs of DNA, and eventually it was shown that the abnormal gene

was present in something between 60% and 75% of all boys affected by Duchenne/Becker dystrophy²⁷ and the long debate as to whether Duchenne and Becker were in fact allelic, was at long last resolved.²⁸ Using amplification techniques (PCR-polymerase chain reaction^{29,30}) it became possible to find deletions in 98% of cases,^{31,32} the DNA being obtained in some instances by using old Guthrie spots.³³ The area of the missing gene in the affected boys was identified in the normal and the protein produced by that segment of DNA was identified and has been called dystrophin.^{34,35} It was then appreciated that the gene which in the normal codes for dystrophin is made up of 70 exons (segments of DNA which are eventually translated into proteins or enzymes) with large numbers of introns (areas of the gene which have at present no known function and are thought to be junk DNA!).^{36,37} It rapidly became recognized that the deletions in the exons were commonly associated with Duchenne/Becker dystrophy³⁸ which by this stage had become known as the Xp21 myopathies.^{39,40}

The implications of the advance to that level were major from the point of view of genetic testing⁴¹ and it opened up the ability to look at fetal tissue at 9 weeks by chorionic villous sampling,⁴²⁻⁴⁴ and to identify whether a male fetus was carrying the abnormal gene. Subsequently it became possible to look for the gene product on routine muscle biopsy samples where dystrophin is completely missing from muscle membrane in Duchenne dystrophy and shows variable expression in Becker dystrophy.^{45,46} The next phase was to explain the clinical differences between the severe form of the disease, Duchenne (where death occurs by the early 20s) compared to the milder form of the disease, Becker (where patients can be wheelchair confined from mid-teens or be ambulant by the age of 50,^{47,48} or even present only with muscle pain on exertion).⁴⁹ It has been shown that if exons are deleted in such a pattern that the ability to read along the gene from the 3 prime end was uninterrupted, then an abnormal or truncated form of dystrophin was produced leading to the milder form of the disease whereas if the deletion occurred in such an area that the ability to read sequentially along the gene was destroyed, then no dystrophin was produced and the severe form of the disease developed clinically.^{50,51}

It rapidly became apparent that disorders which appeared either phenotypically⁵²⁻⁵⁴ or pathologically^{55,56} to be distinct from Becker disease were in fact related to a deletion at the Xp21 site and therefore we are now in the situation where any isolated male who presents with a neuromuscular complaint must be considered to be at risk of having an Xp21 myopathy (dystrophinopathy) until proven otherwise.

We are just starting to move into the field of effective therapy in Duchenne. It has been shown that myoblast transfer⁵⁷⁻⁶¹ (the introduction of normal myoblast from a donor into the affected muscle) will produce within the muscle the missing protein (dystrophin). More recently it has been shown⁶² in the animal model that by using constructs of the gene these can be injected via a vector directly into the muscle and this will be incorporated into the abnormal membrane.⁶³ This opens up the amazing prospect of potential therapy of what used to be one of the most feared diseases in medicine.

In addition to the work which has been carried out on the nuclear DNA, disorders associated with mitochondrial DNA have now been identified,⁶⁴⁻⁶⁷ where abnormalities in the maternally inherited mitochondrial DNA have been shown to occur in Leber's hereditary optic atrophy⁶⁸ and in many forms of chronic progressive external ophthalmoplegia⁶⁹⁻⁷⁶ (CPEO/

Kearns-Sayers syndrome) and substitutions or translocations within the mitochondrial gene have been associated with MELAS⁷⁷⁻⁷⁹ (mitochondrial myopathy with lactic acidosis and stroke-like episodes) and MERRF^{80,81} (myoclonic epilepsy with ragged red fibres).

The possibility that this explosion of knowledge will spill over into more common disorders is raised by the finding of gene markers in some forms of epilepsy (juvenile myoclonic⁸² and in the mouse),³⁰ and the prospect that there may be susceptible gene(s) in multiple sclerosis.⁸³ The rate of increasing information in these fields is exponential and unfortunately, because of this, many physicians are going to be 'left behind' because of the apparent impenetrability of the language used. However, the basic principles are simple and the physician should not be deterred from a close involvement with the field because of its apparent 'high tech' scientific connotations.

References

- Worton, R.G., Bulman, D.E., Zubrzycka-Gaarn, E.E. & Ray, P.N. Genetic and biochemical determinations in the pre-transplant workup and in the post-transplant assessment period. *Adv Exp Med Biol* 1990, **280**: 219-226.
- Dequard-Chablat, M. Translation, oncogenesis and myopathies. *Trends Genet* 1991, **7**: 240-241.
- Wexler, N.S., Rose, E.A. & Housman, D.E. Molecular approaches to hereditary diseases of the nervous system: Huntington's disease as a paradigm. *Ann Rev Neurosci* 1991, **14**: 503-529.
- Deufel, T. & Gerbitz, K.-D. Biochemistry and molecular genetics of muscle diseases. *J Clin Chem Clin Biochem* 1991, **29**: 13-38.
- Rosenberg, R.N. The triumph of linkage analysis. *Ann Neurol* 1990, **27**: 111-112.
- Bailey, J., MacKenzie, A.E., Leblond, S. & Korneluk, R.G. Assessment of a creatine kinase isoform M defect as a cause of myotonic dystrophy and the characterization of two novel CKMM polymorphisms. *Hum Genet* 1991, **86**: 457-462.
- Harley, H.G., Walsh, K.V., Rundle, S. *et al.* Localisation of the myotonic dystrophy locus to 19q13.2-19q13.3 and its relationship to twelve polymorphic loci on 19q. *Hum Genet* 1991, **87**: 73-80.
- Defesche, J.C., Hoogendijk, J.E., De, V.M., Ongerboer, de V.B.W. & Bolhuis, P.A. Genetic linkage of hereditary motor and sensory neuropathy type I (Charcot-Marie-Tooth disease) to markers of chromosomes 1 and 17. *Neurology* 1990, **40**: 1450-1453.
- McAlpine, P.J., Feasby, T.E., Hahn, A.F. *et al.* Localization of a locus for Charcot-Marie-Tooth neuropathy type Ia (CMT1A) to chromosome 17. *Genomics* 1990, **7**: 408-415.
- Cawthorn, R.M., O'Connell, P., Buchberg, A.M. *et al.* Identification and characterization of transcripts from the neurofibromatosis 1 region: the sequence and genomic structure of EV12 and mapping of other transcripts. *Genomics* 1990, **7**: 555-565.
- Fontaine, B., Rouleau, G.A., Seizinger, B.R. *et al.* Molecular genetics of neurofibromatosis 2 and related tumors (acoustic neuroma and meningioma). *Ann NY Acad Sci* 1991, **615**: 338-343.
- Menon, A.G., Ponder, B.A.J. & Seizinger, B.R. The neurofibromatosis genes: from molecular cloning to cellular function. *Cancer Cell* 1991, **3**: 147-152.
- Ponder, B. Human genetics: Neurofibromatosis gene cloned. *Nature* 1990, **346**: 703-704.
- Gusella, J.F., Wexler, N.S. & Conneally, P.M. A polymorphic DNA marker genetically linked to Huntington's disease. *Nature* 1983, **306**: 234-238.
- Harper, P.S. Molecular genetic approaches to Huntington's disease. In: Harper, P.S. (ed.) *Huntington's Disease. Major Problems in Neurology*, ed. v. 22. Saunders, London, 1991, pp. 317-336.
- Farrer, L.A., Myers, R.H., Connor, L., Cupples, L.A. & Growdon, J.H. Segregation analysis reveals evidence of a major gene for Alzheimer disease. *Am J Hum Genet* 1991, **48**: 1026-1033.
- Haines, J.L. Invited editorial: The genetics of Alzheimer disease-A teasing problem. *Am J Hum Genet* 1991, **48**: 1021-1025.
- Heston, L.L., Orr, H.T., Rich, S.S. & White, J.A. Linkage of an Alzheimer disease susceptibility locus to markers on human chromosome 21. *Am J Med Genet* 1991, **40**: 449-453.
- Martin, G.M., Schellenberg, G.D., Wijsman, E.M. & Bird, T.D. Alzheimer's disease: dominant susceptibility genes. *Nature* 1990, **347**: 124.
- Percy, M.E., Markovic, V.D., Crapper, M.L.C.R. *et al.* Family with 22-derived marker chromosome and late-onset dementia of the Alzheimer type: I. Application of a new model for estimation of the risk of disease associated with the marker. *Am J Med Genet* 1991, **39**: 307-313.
- Pericak-Vance, M.A., Bebout, J.L., Gaskell, P.C., Jr *et al.* Linkage studies in familial Alzheimer disease: Evidence for chromosome 19 linkage. *Am J Hum Genet* 1991, **48**: 1034-1050.
- Harrison, P.J. Alzheimer's disease and the β amyloid gene. *Br Med J* 1991, **302**: 1478-1479.
- Siddique, T., Figlewicz, D.A., Pericak-Vance, M.A. *et al.* Linkage of a gene causing familial amyotrophic lateral sclerosis to chromosome 21 and evidence of genetic-locus heterogeneity. *N Engl J Med* 1991, **324**: 1381-1384.

24. Grimm, T., Danieli, G.A. & Muller, C.R. Theoretical expectations for deletional mutations in Duchenne muscular dystrophy. *Am J Med Genet* 1988, **29**: 445–452.
25. Rowland, L.P. Clinical concepts of Duchenne muscular dystrophy. The impact of molecular genetics. *Brain* 1988, **111**: 479–496.
26. Hoffman, E.P., Brown, B.H., Jr & Kunkel, L.M. Dystrophin: the protein product of Duchenne muscular dystrophy gene. *Cell* 1987, **51**: 919–928.
27. Dubowitz, V. The Duchenne dystrophy story: from phenotype to gene and potential treatment. *J Child Neurol* 1989, **4**: 240–250.
28. Fischbeck, K.H. The difference between Duchenne and Becker dystrophies – Editorial. *Neurology* 1989, **39**: 584–585.
29. Carman, W.F. The polymerase chain reaction. *Q J Med* 1991, **78**: 195–203.
30. Rise, M.L., Frankel, W.N., Coffin, J.M. & Seyfried, T.N. Genes for epilepsy mapped in the mouse. *Science* 1991, **253**: 669–673.
31. Beggs, A.H. & Kunkel, L.M. Improved diagnosis of Duchenne/Becker muscular dystrophy. *J Clin Invest* 1990, **85**: 613–619.
32. Beggs, A.H., Koenig, M., Boyce, F.M. & Kunkel, L.M. Detection of 98% of DMD/BMD gene deletions by polymerase chain reaction. *Hum Genet* 1990, **86**: 45–48.
33. Schwartz, E.I., Khalchitsky, S.E., Eisensmith, R.C. & Woo, S.L.C. Polymerase chain reaction amplification from dried blood spots on Guthrie cards. *Lancet* 1990, **336**: 639–640.
34. Kunkel, L.M. Duchenne muscular dystrophy: identification of the gene. In: Rowland, L.P., Woods, D.S., Schon, E.A. & DiMauro, S. (eds) *Molecular Genetics in Diseases of Brain, Nerve, and Muscle*. Oxford University Press, Oxford, 1989, pp. 370–379.
35. Kunkel, L.M. & Hoffman, E.P. Duchenne/Becker muscular dystrophy: a short overview of the gene; the protein; and current diagnostics. *Br Med Bull* 1989, **45**: 630–643.
36. Darras, B.T. Molecular genetics of Duchenne and Becker muscular dystrophy. *J Pediatr* 1990, **117**: 1–15.
37. Love, D.R., Forrest, S.M., Smith, T.J. *et al.* Molecular analysis of Duchenne and Becker muscular dystrophies. *Br Med Bull* 1989, **45**: 659–680.
38. Bartlett, R.J., Percak-Vance, M.A., Koh, J. *et al.* Duchenne muscular dystrophy: high frequency of deletions. *Neurology* 1988, **38**: 1–4.
39. Bieber, F.R. & Hoffman, E.P. Duchenne and Becker muscular dystrophies: genetics, prenatal diagnosis, and future prospects. *Clin Perinatol* 1990, **17**: 845–865.
40. Brown, R.H., Jr & Hoffman, E.P. Molecular biology of Duchenne muscle dystrophy. *Trends Neurosci* 1988, **11**: 480–483.
41. Darras, B.T., Koenig, M., Kunkel, L.M. & Francke, U. Direct method for prenatal diagnosis and carrier detection in duchenne becker muscular dystrophy using the entire dystrophin cDNA. *Am J Med Genet* 1988, **29**: 713.
42. Cole, C.G., Walker, A., Coyne, A. *et al.* Prenatal testing for Duchenne and Becker muscular dystrophy. *Lancet* 1988, **i**: 262–265.
43. Peinemann, F., Wagner, M., Franke, U., Kulle, M. & Reiss, J. Prenatal deletion detection in a sporadic case of Duchenne muscular dystrophy without genotype information from the affected individual. *Eur J Pediatr* 1991, **150**: 256–258.
44. Evans, M.I., Greb, A., Kunkel, L.M. *et al.* In utero fetal muscle biopsy for the diagnosis of Duchenne muscular dystrophy. *Am J Obstet Gynecol* 1991, **165**: 728–732.
45. Nicholson, L.V.B., Davison, K., Falkous, G. *et al.* Dystrophin in skeletal muscle. I. Western blot analysis using a monoclonal antibody. *J Neurol Sci* 1989, **94**: 125–136.
46. Nicholson, L.V.B., Davison, K., Johnson, M.A. *et al.* Dystrophin in skeletal muscle. II. Immunoreactivity in patients with Xp21 muscular dystrophy. *J Neurol Sci* 1989, **94**: 137–146.
47. Beggs, A.H., Hoffman, E.P., Synder, J.R. *et al.* Exploring the molecular basis for variability among patients with Becker muscular dystrophy: dystrophin gene and protein studies. *Am J Hum Genet* 1991, **49**: 54–67.
48. Covone, A.E., Lerone, M. & Romeo, G. Genotype–phenotype correlation and germline mosaicism in DMD/BMD patients with deletions of the dystrophin gene. *Hum Genet* 1991, **87**: 353–360.
49. Gospe, S.M., Lazard, R.P., Lava, N.S., Crootscholten, P.M., Scott, M.D. & Fischbeck, K.H. Familial X-linked myalgia and cramps: a non-progressive myopathy associated with a deletion in the dystrophin gene. *Neurology* 1989, **39**: 1277–1280.
50. Malhotra, S.B., Hart, K.A., Klamut, H.J. *et al.* Frame-shift deletions in patients with Duchenne and Becker muscular dystrophy. *Science* 1988, **242**: 755–758.
51. Koenig, M., Beggs, A.H., Moyer, M. *et al.* The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. *Am J Hum Genet* 1989, **45**: 498–506.
52. Weiss, B.J., Kamholz, J., Ritter, A. *et al.* Segmental spinal muscle atrophy and dermatological findings in a patient with chromosome 18q deletion. *Ann Neurol* 1991, **30**: 419–423.
53. Passos-Bueno, M.R., Terwilliger, J., Ott, J. *et al.* Linkage analysis in families with autosomal recessive limb-girdle muscular dystrophy (LGMD) and 6q probes flanking the dystrophin-related sequence. *Am J Med Genet* 1991, **38**: 140–146.
54. Sunohara, N., Arahata, K., Hoffman, E.P. *et al.* Quadriceps myopathy: forme fruste of Becker muscular dystrophy. *Ann Neurol* 1990, **28**: 634–639.
55. Lunt, P.W., Cumming, W.J.K., Kingston, H. *et al.* DNA probes in differential diagnosis of Becker muscular dystrophy and spinal muscular atrophy [letter]. *Lancet* 1989, **i**: 46.
56. Siddique, T., Roper, H., Percak-Vance, M.A. *et al.* Molecular genetic analysis of a family with an X-linked neuromuscular disorder: spinal muscular atrophy or Becker muscular dystrophy? *Am J Hum Genet* 1988, **43**: A158.
57. Karpati, G. The principles and practice of myoblast transfer. *Adv Exp Med Biol* 1990, **280**: 69–74.
58. Kakulas, B.A. A consideration of therapeutic interventions in the light of the muscle pathology and likely pathogenesis of the XP21.2 muscular dystrophies. In: Kakulas, B.A. & Mastaglia, F.L. (eds) *Pathogenesis and Therapy of Duchenne and Becker Muscular Dystrophy*. Raven Press, New York, 1990, pp. 47–58.
59. Law, P.K., Goodwin, T.G. & Wang, M.G. Normal myoblast injections provide genetic treatment for murine dystrophy. *Muscle Nerve* 1988, **11**: 525–533.
60. Karpati, G. Possible treatment of Duchenne muscular dystrophy by non-dystrophic myoblast implantation into dystrophic muscles. In: Kakulas, B.A. & Mastaglia, F.L. (eds) *Pathogenesis and Therapy of Duchenne and Becker Muscular Dystrophy*. Raven Press, New York, 1990, pp. 59–68.
61. Law, P.K., Bertorini, T.E., Goodwin, T.G. *et al.* Dystrophin production induced by myoblast transfer therapy in Duchenne muscular dystrophy. *Lancet* 1990, **336**: 114–115.
62. Wolff, J.A., Malone, R.W., Williams, P. *et al.* Direct gene transfer into mouse muscle *in vivo*. *Science* 1990, **247**: 1465–1468.
63. Lee, C.C., Pearlman, J.A., Chamberlain, J.S. & Caskey, C.T. Expression of recombinant dystrophin and its localization to the cell membrane. *Nature* 1991, **349**: 334–336.
64. Clarke, A. Mitochondrial genome: defects, disease, and evolution. *J Med Genet* 1990, **27**: 451–456.
65. Harding, A.E. Neurological disease and mitochondrial genes. *TINS* 1991, **14**: 132–138.
66. McKelvie, P.A., Morley, J.B., Byrne, E. & Marzuki, S. Mitochondrial encephalomyopathies: a correlation between neuropathological findings and defects in mitochondrial DNA. *J Neurol Sci* 1991, **102**: 51–60.

67. Palca, J. The other human genome. *Science* 1990, **249**: 1104–1105.
68. Johns, D.R. & Berman, J. Alternative, simultaneous complex I mitochondrial DNA mutations in Leber's hereditary optic neuropathy. *Biochem Biophys Res Commun* 1991, **174**: 1324–1330.
69. Degoul, F., Nelson, I., Amselem, S. *et al.* Different mechanisms inferred from sequences of human mitochondrial DNA deletions in ocular myopathies. *Nucleic Acids Res* 1991, **19**: 493–496.
70. Collins, S., Dennett, X., Byrne, E. & Marzuki, S. Chronic progressive external ophthalmoplegia in patients with large heteroplasmic mitochondrial DNA deletions: an immunocytochemical study. *Acta Neuropathol (Berl)* 1991, **82**: 185–192.
71. Degoul, F., Nelson, I., Lestienne, P. *et al.* Deletions of mitochondrial DNA in Kearns–Sayre syndrome and ocular myopathies: Genetic, biochemical and morphological studies. *J Neurol Sci* 1991, **101**: 168–177.
72. Lauber, J., Marsac, C., Kadenbach, B. & Seibel, P. Mutations in mitochondrial tRNA genes: a frequent cause of neuromuscular diseases. *Nucl Acids Res* 1991, **19**: 1393–1397.
73. Merelli, E., Selleri, L., Ferrari, S., Sola, P., Colombo, A. & Torelli, G. Mitochondrial DNA deletion in oculoskeletal myopathy. *Eur Neurol* 1991, **31**: 160–163.
74. Ota, Y., Tanaka, M., Sato, W. *et al.* Detection of platelet mitochondrial DNA deletions in Kearns–Sayre syndrome. *Invest Ophthalmol Vis Sci* 1991, **32**: 2667–2675.
75. Trounce, I., Byrne, E., Marzuki, S. *et al.* Functional respiratory chain studies in subjects with chronic progressive external ophthalmoplegia and large heteroplasmic mitochondrial DNA deletions. *J Neurol Sci* 1991, **102**: 92–99.
76. Reichmann, H., Degoul, F., Gold, R. *et al.* Histological, enzymatic and mitochondrial DNA studies in patients with Kearns–Sayre syndrome and chronic progressive external ophthalmoplegia. *Eur Neurol* 1991, **31**: 108–113.
77. Hess, J.F., Parisi, M.A., Bennett, J.L. & Clayton, D.A. Impairment of mitochondrial transcription termination by a point mutation associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 1991, **351**: 236–239.
78. Kobayashi, Y., Momoi, M.Y., Tominaga, K. *et al.* A point mutation in the mitochondrial tRNA Leu (UUR) gene in melas (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes). *Biochem Biophys Res Commun* 1990, **173**: 816–822.
79. Kobayashi, Y., Momoi, M.Y., Tominaga, K. *et al.* Respiration-deficient cells are caused by a single point mutation in the mitochondrial tRNA-Leu (UUR) gene in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). *Am J Hum Genet* 1991, **49**: 590–599.
80. Tanno, Y., Yoneda, M., Nonaka, I., Tanaka, K., Miyatake, T. & Tsuji, S. Quantitation of mitochondrial DNA carrying tRNA^{Lys} mutation in MERRF patients. *Biochem Biophys Res Commun* 1991, **170**, 880–885.
81. Bindoff, L.A., Desnuelle, C., Birch-Machin, M.A. *et al.* Multiple defects of the mitochondrial respiratory chain in a mitochondrial encephalopathy (MERRF): a clinical, biochemical and molecular study. *J Neurol Sci* 1991, **102**: 17–24.
82. Qiao, X. & Noebels, J.L. Genetic and phenotypic heterogeneity of inherited spike-wave epilepsy: two mutant gene loci with independent cerebral excitability defects. *Brain Res* 1991, **555**: 43–50.
83. Charnley, P., Beall, S.S., Concannon, P., Hood, L. & Gatti, R.A. Further localization of a multiple sclerosis susceptibility gene on chromosome 7q using a new T cell receptor beta-chain DNA polymorphism. *J Neuroimmunol* 1991, **32**: 231–240.